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Hypercoagulable states: an algorithmic approach to laboratory testing and update on monitoring of direct oral anticoagulants

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Abstract

Hypercoagulability can result from a variety of inherited and, more commonly, acquired conditions. Testing for the underlying cause of thrombosis in a patient is complicated both by the number and variety of clinical conditions that can cause hypercoagulability as well as the many potential assay interferences. Using an algorithmic approach to hypercoagulability testing provides the ability to tailor assay selection to the clinical scenario. It also reduces the number of unnecessary tests performed, saving cost and time, and preventing potential false results. New oral anticoagulants are powerful tools for managing hypercoagulable patients; however, their use introduces new challenges in terms of test interpretation and therapeutic monitoring. The coagulation laboratory plays an essential role in testing for and treating hypercoagulable states. The input of laboratory professionals is necessary to guide appropriate testing and synthesize interpretation of results.

Key Words Hypercoagulability, Algorithmic approach, Antiphospholipid syndrome, Direct oral anticoagulant, Venous thromboembolism

INTRODUCTION

Hypercoagulability, also known as thrombophilia, describes a group of hereditary and acquired conditions which confer a propensity to develop thrombi in the veins, arteries, or both. Based on current knowledge, antiphospholipid syndrome is the most prevalent hypercoagulable state, followed by factor V Leiden (FVL) mutation, prothrombin gene G20210A mutations, elevated factor VIII, and hyperhomocysteinemia. Less common disorders include deficiencies in antithrombin, protein C, or protein S.

The prevalence of thrombosis is higher in individuals with a personal and/or family history of thrombosis than in the general population. Acquired and hereditary risk factors for thrombophilia are summarized in Table 1 [1, 2]. Although patients with hypercoagulable risk factors are at a great risk for developing a thrombotic event, not all patients with hypercoagulable risk factors will develop clinically relevant thrombosis; conversely, not all patients with thrombosis will have an identifiable hypercoagulable state [2-4].

Diagnostic thrombophilia testing is indicated in patients with idiopathic or recurrent venous thromboembolism (VTE), first VTE at a young age (<40 years), VTE in the setting of a strong family history, VTE in an unusual vascular site (cerebral, hepatic, mesenteric, or renal veins), neonatal purpura fulminans, warfarin-induced skin necrosis, and recurrent pregnancy loss [5, 6].

1. Clinical history and sources of interference

Many assays used in the laboratory evaluation of hypercoagulability are affected by concurrent clinical conditions and medications. Whenever possible, a thorough clinical history including site of thrombosis, previous bleeding or thrombotic events, other medical disorders (i.e., liver, autoimmune, or cardiovascular disease), pregnancy, medications (including but not limited to anticoagulants), and family history should be elicited. These are essential for appropriate test selection and interpretation of results; sources of interference for specific assays are described in the following sections. Tests should be performed *at least 4–6 weeks after an acute thrombotic event or discontinuation of anticoagulant/thrombolytic therapies* including warfarin, heparin, direct thrombin inhibitors (DTIs), direct factor Xa inhibitors, and fibrinolytic agents [1, 4, 5]. If abnormal results are found during acute illness or anticoagulant therapy, testing should be repeated in a new specimen when the patient is stable and after anticoagulant therapy is discontinued.

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Table 1. Acquired and hereditary risk factors for thrombophilia.

Acquired factors	Hereditary factors
Major surgery/trauma	Activated protein C resistance/factor V Leiden
Immobilization (e.g., hip/knee replacement, prolonged cast, stroke, bedridden because of illness)	Prothrombin gene G20210A mutation
Solid or hematologic malignancies	Protein C deficiency[a]
Pregnancy	Protein S deficiency[a]
Oral contraceptives	Antithrombin deficiency[a]
Estrogen replacement therapy	Hyperhomocysteinemia[a]
Antiphospholipid antibody syndrome	Elevated factor VIII activity[a]
Heparin-induced thrombocytopenia	Dysfibrinogenemia[a]
Paroxysmal nocturnal hemoglobinuria	
Obesity	
Nephrotic syndrome	
Smoking	

[a]Can be hereditary or acquired.

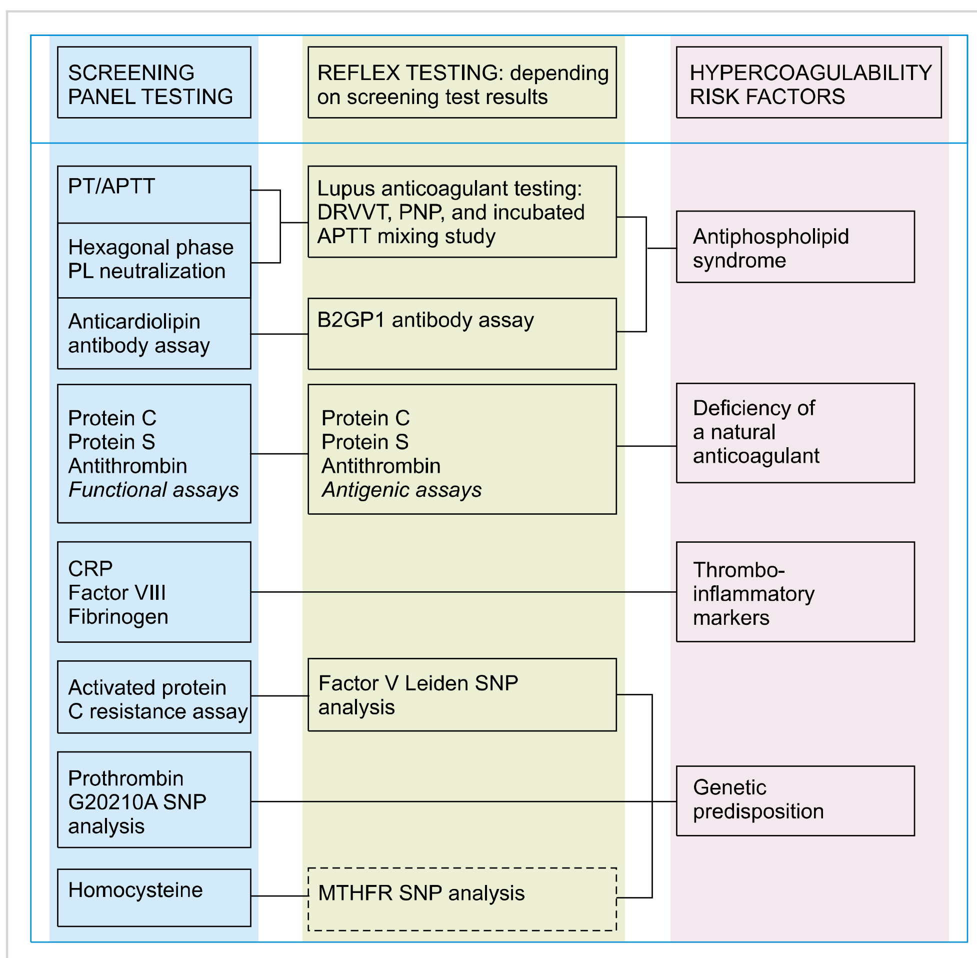

Fig. 1. Comprehensive hypercoagulability testing panel using a diagnostic algorithm. Abbreviations: aPTT, activated partial thromboplastin time; B2GP1, beta2 glycoprotein 1; CRP, C-reactive protein; DRVVT, dilute Russell's Viper Venom test; MTHFR, methylenetetrahydrofolate reductase; PL, phospholipid; PNP, platelet neutralization procedure; PT, prothrombin time; SNP, single nucleotide polymorphism.

Alternatively, thrombophilia testing may be delayed until acute clinical conditions have subsided. The exception is DNA analysis for genetic mutations, which is not generally affected by other medical issues or anticoagulant therapy.

2. Algorithmic approach to laboratory testing

No single laboratory test is yet available that can identify all hypercoagulable defects. Selection of the most informative tests may differ depending on location and type (venous or arterial) of thrombosis. Fig. 1 outlines a testing algorithm to maximize diagnostic potential in patients with thrombophilia while avoiding unnecessary and potentially expensive tests. Testing should be performed in a step-wise manner beginning with high-yield screening tests followed by appro-

priate specific confirmatory tests. These comprehensive panels generate multiple test results that can each be affected by a variety of clinical conditions and drugs. Comprehensive narrative interpretation by coagulation specialists is necessary to synthesize test results, correctly interpret them according to the patient's clinical condition, and provide appropriate guidance to clinicians [2, 7]. In some patients with thrombophilia, it may be best to test for all recognized hereditary risk factors, both common and uncommon [5, 7, 8].

SPECIFIC HYPERCOAGULABLE DISORDERS AND LABORATORY STUDIES

1. Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is the most common cause of acquired thrombophilia. Antiphospholipid antibodies (APAs) are acquired autoantibodies directed against phospholipid-protein complexes and are present in 3–5% of the general population. APAs are associated with increased risk of both arterial and venous thrombosis and recurrent pregnancy loss [9, 10]. APAs can arise spontaneously (primary) or in association with another condition (secondary). Also known as lupus anticoagulants (LA) because of their prevalence in patients with systemic lupus erythematosus (SLE), APAs are extremely heterogeneous and can be directed against a wide variety of anionic phospholipids, including cardiolipin, beta 2 glycoprotein 1 (B2GP1), and cell-membrane phosphatidylserine [3, 11].

Diagnosis of APS requires clinicopathologic correlation because both clinical (either proven vascular thrombosis or pregnancy morbidity) and laboratory criteria must be met. Laboratory diagnostic criteria include positive testing for 1 of the following on 2 or more occasions, at least 12 weeks apart: (1) lupus anticoagulant; (2) anticardiolipin antibodies (IgG or IgM) in medium or high titer; or (3) B2GP1 antibodies (IgG or IgM) in medium or high titer [5, 10-12]. A repeated positive test after a ≥12-week interval is required for diagnosis because transient low-level increases in APA occur in a variety of clinical conditions, including acute phase response, and may not confer increased risk of thrombosis.

1) Lupus anticoagulant testing

Based upon consensus criteria from the International Society for Thrombosis and Haemostasis (ISTH), confirmation of LA requires that the following 4 criteria should be met [11, 13]. (1) Prolongation of at least 1 phospholipid-dependent clotting test (e.g., activated partial thromboplastin time [aPTT], dilute Russell Viper Venom Test [DRVVT] screen or hexagonal phospholipid neutralization screen; assays are usually performed with low concentrations of phospholipid to improve sensitivity). (2) Evidence of inhibitory activity in the patient plasma demonstrated by mixing patient plasma with pooled normal plasma (e.g., immediate and incubated mixing study or DRVVT mixing study). (3) Phospholipid dependence of the inhibitor should be demonstrated by shortening of clotting time after addition of more phospholipid (e.g., DRVVT confirmatory ratio, hexagonal phospholipid neutralization ratio, platelet neutralization). (4) Presence of specific factor inhibitors (especially factor VIII inhibitors) and anticoagulant drugs (heparin or DTI) should be excluded [10, 11, 13-15]. In addition to the ISTH guideline (2009) and updated British Committee for Standards in Haematology (2012) guidelines, the Clinical and Laboratory Standards Institute recently published the first LA guideline [11, 16, 17]. Although all guidelines aim to standardize and harmonize methodologies and improve the quality of LA testing, identifying LA remains a diagnostic challenge.

Paradoxically, LAs prolong clot-based assays *in vitro* while predisposing to thrombosis *in vivo*. In fact, approximately 30% of LA patients will experience thrombosis. In approximately 15% of patients with deep vein thrombosis (DVT), clotting is attributable to LA [2, 18]. Because no single test is available to detect LA, laboratory testing for LA consists of a panel of assays following a diagnostic algorithm (Fig. 1). To maximize diagnostic potential, at least 2 assays based on different principles should be performed to evaluate for each of the 4 ISTH criteria. If fewer than 4 diagnostic criteria are met but clinical suspicion for LA exists, the panel is interpreted as indeterminate and should be repeated at a later date [9-11, 19].

Acute thrombotic events or acute phase responses with elevated factor VIII can cause false-negative results. Thrombin time and anti-Xa assays can help to identify anticoagulant effect or specific inhibitors. Commercially available heparin neutralizers can quench heparin concentrations up to 1.0 U/mL; however, similar reagents are not available for DTIs or specific factor Xa inhibitors. Therefore, LA testing should not be performed on individuals taking these drugs. Individuals on long-term vitamin K antagonist therapy should be tested at least 1–2 weeks after discontinuation of therapy and after the international normalized ratio has normalized to <1.5.

Updated guidelines for LA detection emphasize patient selection to minimize inappropriate requests of LA testing that might lead to false positives. There are 3 grades of appropriate LA testing determined by clinical characteristics. (1) Low grade includes venous or arterial thromboembolism in elderly patients. (2) Moderate grade includes prolonged aPTT in asymptomatic patients, recurrent spontaneous early pregnancy loss, and provoked VTE in young patients. (3) High grade includes unprovoked VTE and arterial thrombosis in young patients (<50 years), thrombosis at unusual sites, late pregnancy loss, and thrombosis or pregnancy morbidity in patients with autoimmune disease. Testing for LA in asymptomatic individuals or patients other than those described here is highly discouraged [11].

2) Anticardiolipin and B2GP1 antibody testing

Anticardiolipin antibodies recognize a complex of cardiolipin, a naturally occurring phospholipid, bound to B2GP1 protein. Specific antibodies against cardiolipin and B2GP1 (IgG or IgM) are measured by commercially available solid-phase ELISA. Assays for anticardiolipin antibodies are

generally considered sensitive; however, because the antigen target of anticardiolipin antibodies is a B2GP1-cardiolipin complex, B2GP1 antibody assays are considered more specific than anticardiolipin antibody assays [20-22]. It is recommended that both anticardiolipin and B2GP1 APA assays be performed to maximize sensitivity and specificity. Current guidelines include only medium and high levels of antibodies (>99th percentile or >40 IgG or IgM phospholipid units) as diagnostic criteria to improve the test specificity. Individuals with high-titer IgG anticardiolipin antibodies (>40 IgG phospholipid units) have been found to have a rate of thrombosis of 6.1% per year, compared with 0.95% per year in individuals with no history of thrombosis, 4.3% in patients with SLE, and 5.5% in patients with a history of thrombosis [4, 9, 12].

Positive tests should be repeated after an interval of at least 12 weeks because transient APAs can occur in relation to infection or drugs; these transient APAs are not associated with thrombotic risk [2, 9]. Additionally, false-positive results for anticardiolipin antibodies can be associated with a high level of rheumatoid factor and cryoglobulins [10, 12].

2. Activated protein C resistance and FVL mutation

Protein C is a vitamin K-dependent serine protease primarily synthesized in an inactive form by the liver [3]. Activation of protein C (APC) requires interaction with the thrombin-thrombomodulin-endothelial protein C receptor complex. Activated protein C regulates thrombin generation by degradation of activated coagulation factors Va and VIIIa in the presence of its cofactor protein S [1]. APC resistance (APC-R) is observed in approximately 20% of patients with a first episode of DVT and 50% of familial thrombosis. More than 90% of APC-R patients have a point mutation in the factor V gene, known as FVL mutation [2, 23, 24]. The FVL mutation (G1691A) results in substitution of glutamine for arginine at position 506 (R506Q); this arginine is 1 of the 3 arginine sites (R306, R506, and R679) cleaved by APC [1]. FVL is present in a heterozygous form in approximately 3–5% of the general Caucasian population and is rare in African, Australian, and South Asian populations [2, 25]. The FVL mutation is the most commonly known hereditary risk factor for venous thrombosis (VT); however, risk for arterial thrombosis remains unclear. VT risk is increased 4- to 8-fold in FVL heterozygotes and 80-fold in homozygotes [6, 26]. Risk of pulmonary embolism (PE) may not be as high as risk of DVT [23]. Thrombotic risk is further increased in the presence of a second risk factor: female FVL heterozygotes using oral contraceptives (OCPs) appear to have a 30- to 60-fold increased risk of thrombosis. Other FV mutations occur but are much less common, including FVR2 haplotype (H1299R), FV Liverpool (I359T), FV Cambridge (R306T), and FV Hong Kong (R306G). These affect APC-R and thrombotic risk [27-29].

Acquired APC-R can be caused by the development of autoantibodies against factor V following exposure to bovine thrombin or with untreated hematological malignancies, LAs, pregnancy, OCPs, active thrombosis, elevated factor VIII, and mutations in the factor VIII gene [2, 4].

Laboratory assays for APC-R and FVL mutation include functional assays and genotyping. The functional APC-R assay is based on prolongation of aPTT by degradation of factors Va and VIIIa by exogenously added APC. The ratio of aPTT in patient plasma and normal plasma before and after adding APC is calculated. This ratio in normal individuals is ≥2.0; in FVL heterozygotes, 1.5–2.0; and in homozygotes, <1.5. Each laboratory should determine its own cutoff for an abnormal result [4, 18]. Elevated factor VIII, low protein S (<20%), and causes of prolonged baseline aPTT (heparin, warfarin, DTI, LA, liver dysfunction/low factor levels) can cause a falsely low APC-R ratio. A second-generation assay includes dilution of patient plasma with factor V-deficient plasma (also containing heparin neutralizer) and provides higher sensitivity and specificity. This assay is less affected by active thrombosis, surgery, inflammatory condition, heparin, or warfarin [30].

Identification of FVL mutation as the cause of APC-R is confirmed by DNA analysis such as PCR-restriction fragment length polymorphism or allele-specific PCR genotyping. A non-PCR-dependent, simple microtiter plate-based Invader assay using fluorescence resonance energy transfer detection shows a reliable detection rate for FVL mutation. However, use of specific primers limits this test to detecting only specific mutations (i.e., FVL), and will not detect other FV mutations. In general, a cost-efficient functional APC-R assay is recommended as an initial screen, with DNA analysis for FVL mutation added for confirmation in individuals with abnormal results [31, 32].

3. Prothrombin gene G20210A mutation

The prothrombin gene G20210A mutation is a gain of function mutation. The mutation occurs in an intron near the 3' end of the gene and alters 3' end processing and/or enhances translation efficiency, resulting in increased prothrombin (factor II) levels [33]. However, the exact mechanism of how increased prothrombin gene expression causes hypercoagulability remains unclear. The prothrombin gene G20210A mutation is the second most common hereditary risk factor for VT [1, 2]. Prevalence varies by ethnicity; 2–4% of Europeans carry the mutation, but it is rare in Asians, Native Americans, and Africans [16, 34, 35]. This mutation is present in approximately 1–3% of the general population, 5–10% of patients with VT, and up to 20% of patients with VT from thrombophilic families. Heterozygous individuals show a 3-fold increased VT risk. However, VT risk will be drastically increased when the patients carry additional inherited or acquired risk factors [2, 4, 36].

Genetic testing to detect the G20210A mutation can be performed by PCR-based methods. Newer assays based on various PCR methods coupled with fluorescence polarization methods or the Invader assay can be performed on automated platforms. DNA microarray technology can detect multiple genetic markers simultaneously with a relatively low cost as a single test compared with conventional DNA assays

[32, 33]. However, these tests require expensive equipment and skilled personnel and may require reflex confirmatory assays (sequencing) if there is an ambiguous or atypical result [32].

4. Protein C deficiency

Protein C deficiency occurs in 0.14–0.50% of the general population and 1–3% of patients with VTE [4, 18]. It is inherited in an autosomal dominant fashion; heterozygotes show functional protein C levels of 40-65% of normal and have a 7-fold increase in risk for VT [35]. The first thrombotic event usually presents at 10–50 years of age [4]. Protein C deficiency also carries increased risk for warfarin-induced skin necrosis. Homozygotes are very rare and can present with neonatal purpura fulminans or disseminated intravascular coagulation (DIC).

Protein C assays measure activity (functional) or antigen quantity (immunological). A functional protein C assay, either clot-based or chromogenic, is usually performed first; if the result is low, an antigenic protein C assay is performed to determine whether the protein defect is quantitative (type I) or qualitative (type II). Type I protein C deficiency is characterized by reduced functional activity and antigen levels and is much more common (75–80%) than type II deficiency (20–25%), which is further subdivided into types IIa (24.5%) and IIb (0.5–1.0%). Both IIa and IIb result in reduced activity with normal antigen levels. If only a quantitative antigenic assay is used, type II deficiency cannot be detected [1, 2]. Clot-based functional protein C assays can detect both types I and II deficiencies but can give falsely increased results with anticoagulant therapy, LAs, and FVL mutation, and falsely decreased results with elevated factor VIII levels (particularly >250%) or low protein S. The chromogenic assay is less affected by interfering substances and is more reproducible; however, it only assesses alterations in the activation and active sites of the protein (type IIa defects) and cannot detect defects in other sites (protein S, surface, or substrate binding sites) and therefore can overlook the rarer type IIb deficiencies [4, 7, 18, 37]. Functional assays, either chromogenic or clot-based, are recommended as initial screens with antigenic assays performed if results are abnormally low.

Acquired deficiency is more common than hereditary forms and must be excluded before making a diagnosis of hereditary protein C deficiency. Because protein C is synthesized in hepatocytes and is vitamin K-dependent, both liver dysfunction and vitamin K deficiency (including warfarin therapy) decrease protein C levels. Protein C has a short half-life (6–8 hours), and levels decrease more rapidly than other coagulation proteins (including protein S and antithrombin) in liver disease and warfarin therapy/vitamin K deficiency. Conversely, protein C levels rapidly normalize after discontinuation of warfarin or correction of vitamin K deficiency; however, levels should not be measured for at least 10 days after warfarin discontinuation. Protein C levels are lowered in recent or current thrombosis, DIC, L-asparaginase therapy, and nephrotic syndrome and during the intra- or immediately postoperative period; neonates also have relatively low protein C levels (17–53%). OCP use and pregnancy can increase protein C levels [2, 7, 38]. Abnormal protein C assays should be repeated after any such conditions have resolved to confirm results.

5. Protein S deficiency

Protein S is a vitamin K-dependent glycoprotein that acts as a cofactor to protein C, accelerating proteolysis of factors Va and VIIIa by approximately 2-fold [39]. Approximately 60% of protein S in the plasma is bound noncovalently to C4bBP in plasma with high affinity; the remaining free (unbound) protein S is the predominant active form. Recent studies have shown that protein S also exerts its own anticoagulant activity by direct binding of factors V, VIII, and X, and appears to act as a cofactor for the tissue factor pathway inhibitor, which results in inhibiting tissue factor-mediated factor X activation [39, 40]. Hereditary protein S deficiency is transmitted in an autosomal dominant fashion and occurs in 0.2–0.5% in the general population and in 1–3% of patients with first VT [7, 37, 41]. Functional protein S levels range 20–64% in heterozygous patients [42]. Homozygous patients typically present as newborns with purpura fulminans and DIC.

There are 3 types of protein S deficiency. Types I and III are quantitative deficiencies with both low free protein S antigen and low protein S activity, and account for 95% of cases. Type I deficiency shows low total (free+C4bBP-bound) protein S antigen level, whereas type III shows normal total protein S levels. Type III deficiency may be related to excess binding of protein S to C4bBP. Type II deficiency is a qualitative defect with low protein S activity and normal antigenic (free and total) protein S levels [35, 43, 44].

Functional (clot-based) protein S assays measure activity, and immunologic methods (monoclonal antibody-based enzyme immunoassay and immunoturbidimetric assay) measure antigenic levels of free or total protein S. Clot-based functional assays are sensitive for all types of deficiency and are used by some laboratories as an initial screening test, similar to the algorithm for protein C. However, functional protein S assays are not specific; potential sources of interference include APC-R (usually from FVL mutation) and LAs. Despite the fact that using an antigenic test as an initial assay may miss type II protein S deficiency, some guidelines recommend antigenic tests as a screen with functional tests performed only on abnormal results [45].

As with protein C deficiency, acquired causes of protein S deficiency are more common and should be excluded before making a diagnosis of hereditary deficiency. Protein S is decreased in conditions that decrease protein C (see the previous section). Protein S is also decreased during the acute phase response because C4bBP is an acute phase reactant; increased C4bBP lowers both protein S activity and free antigen. Protein S is decreased with elevated factor VIII (>250%), and infectious and autoimmune conditions such as HIV infection and inflammatory bowel disease [4, 18, 41]. Levels are usually lower in women, especially during

hormone replacement therapy, OCP use, and the second or third trimester of pregnancy [1]. Abnormal results should be confirmed after any such conditions are resolved.

6. Antithrombin deficiency

Antithrombin (half-life: 2–3 days) is a glycoprotein of the serine protease inhibitor (serpin) family that primarily inactivates activated thrombin (factor IIa) and factor Xa, and to a lesser extent, factors IXa, XIa, and XIIa. Antithrombin acts as a "suicide inhibitor" by forming 1:1 covalent complexes between antithrombin and serine proteases; inhibitor activity is greatly accelerated by interaction with heparin. Although synthesized in the liver parenchyma, antithrombin is not vitamin K-dependent [46, 47].

Antithrombin deficiency is inherited in an autosomal dominant fashion and associated almost exclusively with VT. Prevalence rates are approximately 0.05–0.1% in the general population. Estimated annual incidence of a first episode of VTE in heterozygotes is 1.0–2.9% annually in retrospective studies [18, 47]. Risk of thrombosis appears to be higher in antithrombin deficiency than protein C or protein S deficiency, APC-R, or prothrombin gene G20210A mutation and thus has the highest VTE risk among known hereditary thrombophilias. The homozygous state is almost universally fatal in utero. Functional antithrombin levels in heterozygous individual ranges 35–70% [4, 8, 48, 49]. Besides presenting as DVT or PE, VTE from antithrombin deficiency can occur in unusual sites, such as cerebral sinuses and the mesenteric, portal, and renal veins. The first event occurs at a young age (<50 years) but is uncommon during the first 2 decades of life, and may or may not follow a provocative event. Approximately 58% of first events occur spontaneously, whereas 42% are associated with a transient, potentially preventable, risk factor. Patients with concurrent defects such as FVL mutation are associated with higher risk of VTE at younger ages (median: 16 years) [41, 50].

Antithrombin assays measure both functional activity (usually by chromogenic methods) and antigen quantity (enzyme immunoassays and immunoturbidimetric methods). If the functional level is normal or elevated, antithrombin deficiency is unlikely. Low results should be confirmed, and both functional and antigenic levels should be tested on the new specimen to determine the type of deficiency [4, 18].

Type I antithrombin deficiency is a quantitative defect showing proportionately reduced (approximately 50%) functional and antigenic levels. Type II deficiency is a qualitative defect, resulting in lower activity than antigen. Type II deficiencies are further classified by antithrombin mutation site. However, subclassification is generally not clinically necessary because anticoagulant therapy does not differ between types [41, 46, 47, 49].

Acquired antithrombin deficiency must be excluded before making a diagnosis of hereditary deficiency and can be caused by drugs such as heparin or L-asparaginase. Other causes of low antithrombin include reduced synthesis (liver disease) or increased loss in nephrotic syndrome, protein losing enteropathy, DIC, sepsis, burn, trauma, hepatic venoocclusive disease, thrombotic microangiopathies, cardiopulmonary bypass surgery, hematomas, or metastatic tumors. Activity can be reduced by up to 30% during full-dose unfractionated heparin therapy, but not low-molecular-weight heparin therapy, and levels normalize when heparin is discontinued. Antithrombin can also be low in premenopausal women, OCP use, and pregnancy. As with low protein C and protein S, confirmatory testing should be repeated on a new specimen after any potential confounding conditions have resolved [4, 7, 48, 51].

7. Hyperhomocysteinemia

Homocysteine is an intermediate amino acid produced by demethylation of methionine via methylenetetrahydrofolate reductase (MTHFR) in the folate cycle. Homocysteine metabolism requires vitamin B6, vitamin B12, and folate. Hyperhomocysteinemia is associated with increased risk of VTE, coronary heart disease, acute myocardial infarction, peripheral artery disease, stroke, aneurysm, migraine, hypertension, male infertility, risk for offspring with neural tube defect, and recurrent pregnancy loss. Acquired hyperhomocysteinemia can be caused by vitamin B6, vitamin B12, or folate deficiency; renal failure; hypothyroidism; rheumatoid arthritis; and certain drugs such as methotrexate, niacin, anticonvulsant, theophylline, L-dopa, thiazide, cyclosporine A, or phenytoin [4, 52, 53].

Hereditary hyperhomocysteinemia is caused by polymorphisms in an enzyme necessary in homocysteine conversion pathways. Homozygous alterations of the *MTHFR* gene are present in 10–13% of the population, whereas heterozygous alterations are found in 30–40% [52]. Two commonly recognized polymorphic variants in the *MTHFR* gene are (1) the "thermolabile" c.665C->T (p.Ala222Val), historically referred to as c.677C->T, and (2) c.1286A->C (p.Glu429Ala), also known as c.1298A->C. Meta-analyses of 2 common polymorphisms have found a weak association with risk of thrombosis [7, 54-56]. Homozygosity for *MTHFR* c.665C->T is associated with an approximately 25% increase in plasma homocysteine. Patients with elevated homocysteine and the *MTHFR* c.665C->T and/or c.1286A->C mutations, however, may be at mildly increased risk for both VTE (odds ratio: 1.27) and recurrent pregnancy loss (pooled risk: 2.7) [56]. Lowering homocysteine levels using vitamin B6, vitamin B12, or folate has not been proven to reduce thrombotic risk. Given the modest increase in thrombophilic risk and lack of evidence of therapeutic benefit, screening homocysteine levels in healthy individuals and testing for *MTHFR* polymorphisms are not currently suggested [4, 38, 56].

8. Elevated factor VIII

Several studies have demonstrated an association between elevated factor VIII and increased risk of thrombophilia, partly due to factor VIII-mediated enhancement of thrombin generation [57-60]. Although no genetic variations in the factor VIII gene have been identified, levels appear to be

higher in African-Americans and lower in individuals with blood group O. Factor VIII can be elevated during acute phase reactions, elevated estrogen, pregnancy, or after aerobic exercise. The prevalence of elevated factor VIII among patients with VT is 20–25% [57, 60, 61]. Whether factor VIII elevation directly contributes to increased thrombophilic risk remains unclear; however, studies show that persistent factor VIII level >150% (or >90th percentile) in the absence of acute phase reactions, elevated estrogen levels, or recent exercise is an independent risk factor for thrombophilia [61-63].

Factor VIII activity can be measured by aPTT-based clotting assay or chromogenic assay, and antigen quantitation can be accomplished using ELISA. Factor VIII measurement should be postponed until at least 6 months after an acute thrombotic event and 6 weeks after giving birth and should be repeated after 3–6 months to confirm persistent elevation [2, 63].

9. Fibrinogen defects

Dysfibrinogenemia describes a heterogeneous group of disorders resulting in structurally and functionally altered fibrinogen. It can cause bleeding, venous or arterial thrombosis, or both. The prevalence of dysfibrinogenemia in patients with VT is approximately 0.8% [64-66]. Although the exact mechanism of thrombosis is unknown, it may be related to increased fibrin formation and/or impaired fibrinolysis. Patients with dysfibrinogenemia may have prolonged prothrombin time (PT), thrombin time, and reptilase time; decreased functional fibrinogen; and normal to elevated immunologic fibrinogen. The ratio of functional fibrinogen activity to immunologic fibrinogen antigen will be decreased [2, 7, 66, 67].

The most commonly used functional assay is the Clauss method. Acquired fibrinogen deficiency can be caused by liver disease, consumptive states such as placental abruption or DIC, or fibrinolytic therapy. Because fibrinogen is an acute phase reactant, testing should be delayed at least 6 months after acute thrombosis [67].

MONITORING DIRECT ORAL ANTICOAGULANTS

Warfarin has been the only orally administered anticoagulant and thus the mainstay of outpatient management of patients with hypercoagulable states. Although effective, warfarin has multiple interactions with medications and food and variable pharmacogenetics that necessitate routine monitoring. Patients appear to spend only a fraction of time in the therapeutic range, leaving them at risk both for thrombosis and bleeding [68]. Recently, new direct oral anticoagulants (DOACs) have been introduced, including the direct thrombin inhibitor dabigatran and direct factor Xa inhibitors rivaroxaban and apixaban, which have more predictable pharmacodynamics and pharmacokinetics than warfarin. They are also characterized by a rapid onset of anticoagulant activity and fixed dosing with peak blood concentrations 2–4 hours after ingestion. Notably, none currently has an antidote or specific reversal agent [69]. These drugs were initially shown to be noninferior to warfarin for prevention of stroke and systemic embolism in patients with nonvalvular atrial fibrillation [70-72]. Since then, all 3 have also been approved in the United States for VTE prophylaxis in patients undergoing hip and knee replacement, and dabigatran and rivaroxaban are approved for treatment and reduction of risk of recurrence of DVT and PE [73].

This section briefly describes recommendations for monitoring DOACs. For a more in-depth discussion, readers are directed to the published ISTH Guidelines [74-76] and recent reviews by Drs. Eby and Mani, Kasper, and Lindhoff-Last [68, 69, 77]. These reviews also describe the influence of DOACs on other coagulation assays, including LA testing, which is not covered here.

Although most patients being treated with DOAC do not require monitoring, there are certain clinical scenarios in which measurement of drug levels is necessary. These include episodes of bleeding, before surgery or invasive procedure and perioperative management, concomitant use of drugs with known DOAC interactions, body weight extremes, renal impairment, suspected overdose or noncompliance, and DOAC treatment failure (thrombosis during therapy) [74]. For emergent situations, especially if drug history is not known, or at peak drug levels, a qualitative or semi-quantitative assay will often suffice. However when dose adjustment is necessary (e.g., during treatment failure, renal impairment), a quantitative assay is required [68, 74].

Although dabigatran and factor Xa inhibitors influence routine PT and aPTT, the effects are variable, with dabigatran exerting more influence on aPTT and factor Xa inhibitors prolonging PT. Moreover, the degree of prolongation is highly dependent on the reagent used for the assay. These widely available tests can be used to detect peak or supratherapeutic drug levels, but should not be used for quantitation. They may also appear normal during low or trough drug levels. Each laboratory should be aware of how its specific PT and aPTT reagents behave in the presence of these drugs. This can be achieved using commercially available calibrators [68, 69, 74].

Because of its mechanism of action, the thrombin time (TT) is exquisitely sensitive for dabigatran, and a normal TT essentially excludes the presence of the drug. The effect on TT is linear, but for monitoring near the therapeutic range, a diluted assay is necessary [74]. The ecarin clotting time or chromogenic anti-IIa assays can also be used to quantitate DTI effect; however, these tests are not widely available [68, 78].

For anti-Xa inhibitors, the PT can be used as a qualitative assay; in addition, the STA Neoplastin Plus reagent (Diagnostica Stago, Asnieres, France) can reportedly be used for quantitative measurement of rivaroxaban [75]. However, the PT is generally less sensitive for apixaban than rivaroxaban [76]. Anti-Xa assays, chromogenic or clot based, are widely available because they are used in heparin and hep-

Table 2. The recommended assays for different types of DOAC monitoring.

	Dabigatran	Rivaroxaban and Apixaban
Qualitative/semiquantitative for high levels	aPTT with a sensitive reagent	PT with a sensitive reagent
Highly sensitive screen	TT	Anti-Xa
Quantitative (using appropriate calibrators)	Diluted TT, factor IIa, Ecarin clotting time	Anti-Xa

Abbreviations: aPTT, activated partial thromboplastin time; DOAC, direct oral anticoagulant; PT, prothrombin time; TT, thrombin time.

arin-like drug monitoring. These assays can be used to quantitatively and sensitively measure factor Xa inhibitor effects. Although interlaboratory variability has been reported in the past, commercially available calibrators are now available to aid in standardization [75-77]. Table 2 summarizes the recommended assays for different types of DOAC monitoring.

CONCLUSION

Laboratory evaluation of hypercoagulable patients is often complicated, but it is essential for diagnosis and monitoring therapy. Knowledge of the clinical conditions that cause hypercoagulability, the assays required to detect them, and potential testing interferences is required for appropriate test selection and interpretation.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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